

ORIGINAL ARTICLE

Pericardial Fluid and Serum Levels of Vascular Endothelial Growth Factor and Endostatin in Patients With or Without Coronary Artery Disease

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Background/Purpose: Vascular endothelial growth factor (VEGF) and endostatin are related to ischemic heart disease. This study investigated pericardial fluid and serum levels of VEGF and endostatin in patients with or without ischemic heart disease.

Methods: A total of 39 patients (24 patients in the CAD group with significant coronary artery disease; 15 patients in the non-CAD group without coronary artery disease) undergoing open heart surgery were enrolled. In the CAD group, patients were classified according to good coronary collateralization (Group A; $n = 11$) or poor coronary collateralization (Group B; $n = 13$). Pericardial fluid and serum samples were obtained at the time of surgery. VEGF and endostatin were measured by enzyme-linked immunosorbent assay.

Results: The levels of endostatin in both serum and pericardial fluid were significantly lower in the CAD group than in the non-CAD group (130.5 ± 37.3 ng/mL vs. 172.4 ± 37.8 ng/mL and 119.0 ± 25.0 ng/mL vs. 143.0 ± 23.5 ng/mL). The concentration of serum VEGF in the CAD group (92.6 ± 18.2 pg/mL) was significantly higher than that in the non-CAD group (75.2 ± 22.3 pg/mL). The concentration of serum VEGF in Group A (100.1 ± 20.7 pg/mL) was significantly higher than that in Group B (84.3 ± 12.4 pg/mL). The levels of pericardial fluid VEGF, serum and pericardial fluid endostatin were not significantly different between Groups A and B.

Conclusion: Patients with coronary artery disease have lower serum and pericardial fluid levels of endostatin and higher serum levels of VEGF. Serum level VEGF, but not endostatin, is associated with good or poor collateralization in patients with coronary artery disease. [*J Formos Med Assoc* 2006;105(5):377–383]

Key Words: collateral circulation, coronary artery disease, endostatin, vascular endothelial growth factor

The heart is not only a target of neurohormonal activity, but also an active endocrine and paracrine organ.¹ The cardiac tissue produces many physiologically active substances such as cytokines, growth factors and cardiac hormones.² These secreted substances may be carried out into the pericardial space.² Therefore, analysis of pericardial

fluid provides a modality to elucidate the pathophysiology of the heart. Endostatin is a potent endogenous angiogenesis inhibitor and an effective inhibitor of tumor growth in animal models.^{3–7}

Endostatin exhibits strong antiangiogenic activity by inhibiting proliferation and migration of endothelial cells, in addition to inducing endo-

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thelial cell apoptosis.^{3,8} Elevated serum endostatin is associated with tumor vascularity and poor outcome in a variety of malignancies.⁹⁻¹¹

Vascular endothelial growth factor (VEGF) is an angiogenic growth factor that is implicated in both physiologic and pathologic angiogenesis.^{12,13} It is elevated in patients with acute myocardial infarction and coronary heart disease.^{14,15}

Patients with ischemic heart disease or coronary artery disease (CAD) have reduced pericardial levels of endostatin.¹⁶ It is not known whether serum levels of endostatin correlate with pericardial endostatin in these patients with ischemic heart disease. Furthermore, previous studies have not investigated the pericardial level of endostatin in patients with non-ischemic heart disease. This study investigated both serum and pericardial levels of endostatin and VEGF in patients with or without ischemic heart disease.

Methods

Patients

This study included 39 patients undergoing open heart surgery. The CAD group consisted of 24 patients with ischemic heart disease undergoing coronary artery bypass grafting (CABG), and the non-CAD group consisted of 15 patients with non-ischemic heart disease. This study was approved by the institutional review board of this hospital. All subjects gave written informed consent. Patients with a prior history of CABG, evidence of ongoing malignancy or neoplasm, or infection were excluded.

Coronary angiography

All patients were referred for selective coronary angiography within 1 month before open heart surgery for evaluation of coronary lesions and collateral circulation. A significant coronary stenosis was defined as > 70% narrowing of a major coronary artery branch or > 50% narrowing of the left main coronary artery. Collateral circulation was graded on a scale of 0 to 3 as described by Rentrop et al.¹⁷ In the CAD group, patients were further clas-

sified according to the presence of coronary collateralization of grade 2 and 3 (Group A, good collateral circulation) or of grade 0 and 1 (Group B, poor collateral circulation). Two observers assessed the coronary cineangiograms in a blinded fashion and reached a consensus regarding the collateral filling.

Study protocol

Immediately following incision of the pericardium, undiluted pericardial fluid and blood samples from the brachial vein were obtained prior to heparinization. The pericardial fluid and blood samples were collected in sterile tubes, placed immediately on ice, clarified by centrifugation at 3000g for 10 minutes at 4° C, and rapidly frozen at -80° C. Concentrations of endostatin and VEGF in pericardial fluid and serum were measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). The mean minimum detectable levels for endostatin and VEGF were 23 pg/mL and 5 pg/mL, respectively. Absorbance was measured by optical densitometry at 450 nm.

Data collection and statistical analysis

Data collected preoperatively from medical charts included demographic data and medical history with emphasis on risk factors. Categorical demographic data were compared by Fisher's exact test. Continuous variables were expressed as mean \pm standard deviation, and compared by Student's *t* test and multivariate stepwise logistic regression. A *p* value < 0.05 was considered to be statistically significant.

Results

Among the 24 patients with CAD (22 men, 2 women; mean age, 62.1 \pm 9.3 years), 13 had stable angina and the remaining 11 had acute coronary syndrome. Unstable angina, non-ST segment elevation or ST-segment elevation myocardial infarction was defined as acute coronary syndrome. The demographics and clinical presentations among

patients in the CAD group are summarized in Table 1. No significant difference in patient characteristics and clinical presentations were evident between groups A and B. The number of patients with hyperlipidemia and the use of lipid-lowering therapy (statin) were similar between the two groups. The CAD group comprised patients with either stable angina or acute coronary syndrome. These two syndromes had a similar prevalence in the two groups. The extent of CAD is summarized in Table 2. Patients in Group A had significantly more occluded arteries (100% vs. 31%) and regional wall motion abnormality (73% vs. 23%) than patients in Group B. The number of diseased vessels with > 70% stenosis, ejection fraction, and number of coronary vessels involved were similar in the two groups.

Of the 15 patients in the non-CAD group (10 men, 5 women; mean age, 58.9 ± 14.4 years), five had mitral valve disease, nine had aortic valve disease, and one had atrial septal defect. There was a lower prevalence of coronary risk factors including diabetes (7% vs. 62%), hypertension (7% vs. 62%), hyperlipidemia (7% vs. 58%), and smoking (14% vs. 42%) in the non-CAD group compared with the CAD group.

As shown in Figure 1, the concentration of serum VEGF in the CAD group (92.6 ± 18.2 pg/mL) was significantly higher ($p < 0.05$) than in the non-CAD group (75.2 ± 22.3 pg/mL). The concentration of VEGF in pericardial fluid was similar in both groups (28.0 ± 8.6 pg/mL and 32.2 ± 11.5 pg/mL, respectively; $p = \text{NS}$). In these two groups of patients with or without ischemic heart disease, the

Table 1. Demographics and clinical characteristics of coronary artery disease patients with good (Group A) or poor (Group B) collateral circulation

	Group A (n = 11)	Group B (n = 13)	p
Age (yr)	62.2 ± 8.9	62.1 ± 9.8	NS
Male/female	10/1	12/1	NS
Diabetes (n)	7	8	NS
Hypertension (n)	8	7	NS
Hyperlipidemia (n)	7	7	NS
Smoking (n)	5	5	NS
Previous MI* (n)	6	6	NS
Stable angina (n)	6	7	NS
Unstable angina (n)	2	2	NS
Acute MI (n)	3	4	NS
Enzyme positivity† (n)	3	4	NS
Previous PCI (n)	4	4	NS

*Including unstable angina and myocardial infarction; †elevation in troponin I. MI = myocardial infarction; PCI = percutaneous coronary intervention; NS = not significant.

Table 2. Extent of coronary artery disease in patients with good (Group A) or poor (Group B) collateral circulation

	Group A (n = 11)	Group B (n = 13)	p
Number of diseased vessels > 70%	2.9 ± 0.3	2.9 ± 0.3	NS
Three-vessel disease (n)	10	12	NS
Left main disease > 50%	4	4	NS
Occluded coronary arteries (n)	11	4	0.0006
Regional wall motion abnormality* (n)	8	3	0.038
Ejection fraction % (mean \pm SD)	58.9 ± 9.9	57.9 ± 13.9	NS

*Regional wall motion abnormality indicates presence of hypokinesia, akinesia or dyskinesia of segmental wall of left ventricle during left ventriculography. NS = not significant; SD = standard deviation.

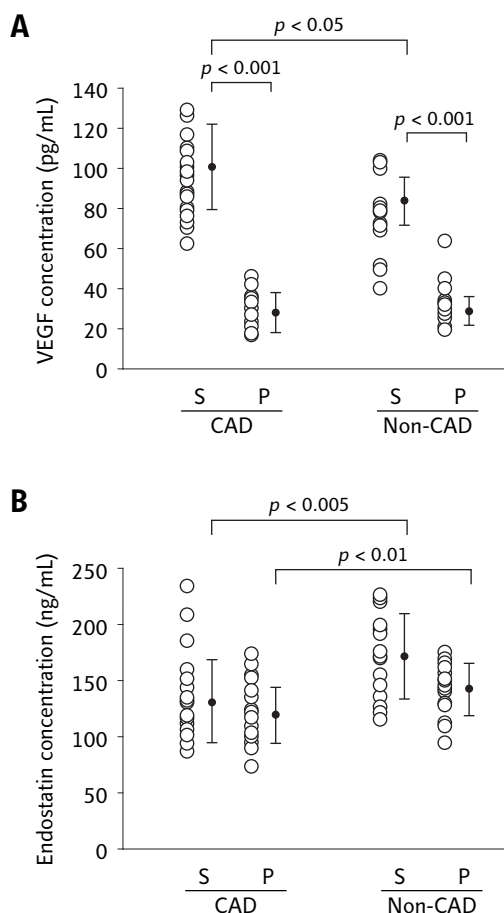


Figure 1. Serum (S) and pericardial fluid (P) levels of: (A) vascular endothelial growth factor (VEGF); and (B) endostatin in coronary artery disease (CAD) and non-CAD patients. Error bars indicate \pm standard deviation.

concentration of VEGF in serum was significantly higher ($p < 0.001$) than in pericardial fluid. The concentration of serum endostatin was significantly lower ($p < 0.005$) in the CAD group (130.5 ± 37.3 ng/mL) than in the non-CAD group (172.4 ± 37.8 ng/mL). The level of endostatin in pericardial fluid was also significantly lower ($p < 0.01$) in the CAD group (119 ± 25 ng/mL) than in the non-CAD group (143.0 ± 23.5 ng/mL). In both the CAD and non-CAD groups, the level of endostatin in serum was not significantly different from that in pericardial fluid.

As shown in Figure 2, the concentration of serum VEGF in Group A (100.1 ± 20.7 pg/mL) was significantly higher ($p < 0.05$) than that in Group B (84.3 ± 12.4 pg/mL). The concentration of VEGF in pericardial fluid was similar in both groups (27.0 ± 10.2 pg/mL and 28.9 ± 7.3 pg/mL, re-

spectively; $p = \text{NS}$). In both of these groups, the concentration of VEGF in serum was significantly higher ($p < 0.001$) than that in pericardial fluid. The level of endostatin in serum and pericardial fluid was similar in both Groups A and B.

There was no correlation between the level of serum VEGF and endostatin ($r = 0.15$, $p = 0.47$). No correlation was found between pericardial fluid VEGF and endostatin ($r = 0.39$, $p = 0.06$). There were no significant differences in serum VEGF or endostatin with respect to gender, diabetes mellitus, hypertension, smoking, acute coronary syndrome, or ejection fraction.

Discussion

This study demonstrated that the concentration of serum VEGF in patients with significant CAD

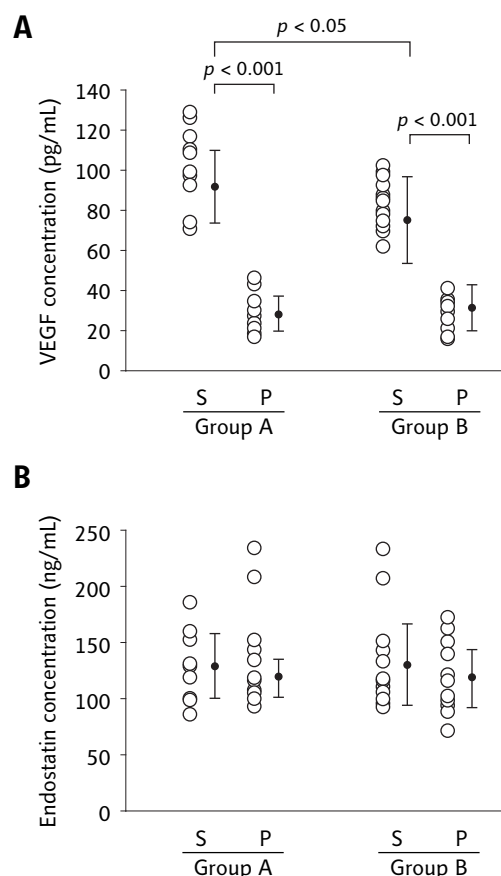


Figure 2. Serum (S) and pericardial fluid (P) levels of: (A) vascular endothelial growth factor (VEGF); and (B) endostatin in Group A and Group B patients with significant coronary artery disease. Error bars indicate \pm standard deviation.

was significantly higher than in patients without CAD. This finding is consistent with a previous report of elevated VEGF in patients with CAD.¹⁸ The concentration of serum VEGF in Group A (patients with good collateral circulation) was significantly higher than that in Group B (patients with poor collateral circulation). The concentration of VEGF in pericardial fluid was similar in these two groups. The level of VEGF in serum was higher than that in pericardial fluid. This finding is also consistent with a previous study.¹⁹ These data indicate that serum VEGF, but not pericardial fluid VEGF, is associated with good or poor collateral circulation in patients with significant CAD. This finding is in agreement with data indicating that VEGF is the most potent angiogenic growth factor for the stimulation of collateral vessel growth in myocardial ischemia.²⁰ VEGF has been shown to be increased in patients with acute myocardial infarction.^{15,21} The number of patients with unstable angina or acute myocardial infarction was similar in Group A and in this study, suggesting that the levels of serum or pericardial VEGF were not affected by these acute conditions. Previous studies have demonstrated the need for transient or permanent coronary vessel occlusion as a stimulus for growth factor modulation and collateral vessel formation.^{22,23} The present study demonstrated a higher prevalence of occluded coronary arteries in Group A patients than in Group B patients, supporting these previous findings.

Alber et al recently reported that plasma VEGF concentrations do not correlate with the presence, severity, and extent of CAD.²⁴ In contrast, increased VEGF concentrations in CAD patients were found in this study and by Blann et al.¹⁸ This discrepancy is probably attributable to different patient populations. Patients with recent acute myocardial infarction were excluded by Alber et al, but were included by Blann et al and in the present study. In addition, the controls in the study of Alber et al were referred for coronary angiography because of chest pain, whereas those in the present study all had documented valvular heart disease.

This study demonstrated that endostatin concentrations in serum and pericardial fluid were

significantly lower in patients with significant CAD than in those without CAD. However, endostatin levels in serum and pericardial fluid were similar in CAD patients with good or poor collateral circulation. Panchal et al demonstrated that pericardial fluid endostatin levels were nearly 40% lower in ischemic heart disease patients with good collateralization compared with poor collateralization.¹⁶ In their study, serum levels of endostatin were not measured and control patients without CAD were not enrolled.¹⁶ The discrepancy between the findings of this study and that of Panchal et al is probably attributable to differences in the patient populations. The prevalence rates of hypertension (60% vs. 85%), smoking status (42% vs. 77%), and hyperlipidemia (58% vs. 85%) were all lower in this study than in Panchal et al's study. It is not known, however, whether these risk factors affect endostatin level. The small sample size and racial differences may also be partially responsible for the discrepancy between these two studies. Our study is the first one to demonstrate lower serum endostatin levels in patients with significant CAD.

Hypoxia and myocardial ischemia are the major stimuli that cause increased VEGF expression.^{12,14} The potent angiogenic properties of VEGF are well described. It has beneficial actions besides inducing new vessel formation.²⁵ VEGF augments several endothelial cell functions, including nitric oxide and prostacyclin production, which have vasodilatory and antiproliferative effects. The elevated VEGF level in patients with significant CAD may compensate for both the endothelial dysfunction and the ischemia in these patients. Other angiogenic growth factors such as fibroblast growth factor and inflammatory cytokines are elevated in patients with ischemic heart disease.^{26,27} Their roles in coronary collateral circulation need further investigation.

Endostatin is a potent inhibitor of angiogenesis.¹³ Angiogenesis is mediated by a balance of proangiogenic and antiangiogenic factors that favor neovascularization.²⁸ Repression of antiangiogenic influences, such as endostatin, may facilitate the process of neovascularization. The pre-

sent study demonstrated that good or poor collateral circulation was not associated with the serum or pericardial fluid levels of endostatin. Neovascularization at the capillary level was not visible on coronary angiography. Therefore, we cannot exclude the possibility that lower endostatin levels in patients with significant CAD may contribute to myocardial angiogenesis.

In summary, patients with significant CAD have lower serum and pericardial fluid levels of endostatin, and higher serum levels of VEGF. Serum VEGF, but not endostatin, is associated with good or poor collateralization in patients with significant CAD.

Acknowledgments

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